

The invention claimed is:

1. A method for separating a mixture of polynucleotides, said method comprising:
applying said mixture of polynucleotides to a polymeric monolith having non-
polar chromatographic surfaces and eluting said mixture of polynucleotides
with a mobile phase comprising a counterion agent and an organic solvent,
wherein said monolith is contained within a fused silica tube having an inner
diameter in the range of 1 micrometer to 1000 micrometer,
wherein said monolith is immobilized by covalent attachment at the inner wall
of said tube, and
wherein said monolith comprises an underivatized poly(styrene-
divinylbenzene) matrix.

2. A method of claim 1 wherein said tube is devoid of retaining frits.

3. A method of claim 1 wherein said monolith is characterized by having 100,000 to
200,000 theoretical plates per meter.

4. A method of claim 3 wherein said theoretical plates per meter is determined from
the retention time of single stranded p(dT)₁₈ standard using the following
equation:

$$(N/L) = (5.54/L) \left(\frac{t_R}{w_{0.5}} \right)$$

wherein N is the number of theoretical plates, t_R is the retention time of said
standard determined during an isocratic elution, $w_{0.5}$ is the peak width at half
height, and L is the length of the monolith in meters.

5. A method of claim 4 wherein said tube has an inner diameter of 200 micrometer
and a length of 60 mm, wherein during said isocratic elution said monolith has
a back pressure in the range of 180 to 200 bar, and a flow rate in the range of
2 to 3 $\mu\text{L}/\text{min}$ at an elution temperature of 50°C.

6. A method of claim 1 wherein said mobile phase is devoid of EDTA.

7. A method of claim 1 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is brush-like.

8. A method of claim 1 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

9. A method for separating a mixture of polynucleotides, said method comprising: applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix, wherein said monolith is contained within a fused silica tube, and wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

10. A method of claim 9 wherein said monolith is contained within said fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer.

11. A method of claim 9 wherein said tube is devoid of retaining frits.

12. A method of claim 9 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

13. A method of claim 9 wherein said mobile phase is devoid of EDTA.

14. A method of claim 9 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

15. A method of claim 9 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

16. A method for separating a mixture of polynucleotides, said method comprising: applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube,

wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer,

wherein said tube is devoid of retaining frits, and

wherein said polynucleotides comprise double-stranded fragments having lengths in the range of 3 to 600 base pairs.

17. A method of claim 16 wherein said mobile phase is devoid of EDTA.

18. A method of claim 17 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

19. A method of claim 16 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

20. A method of claim 16 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

21. A method of claim 16 wherein said monolith is characterized by having at least 100,000 theoretical plates per meter.

22. A method of claim 16 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

23. A method for separating a mixture of polynucleotides, said method comprising:
applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,
wherein said monolith is characterized by having 10,000 to 200,000 theoretical plates per meter,
wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, and

wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

24. A method of claim 23 wherein said theoretical plates per meter is determined from the retention time of single stranded p(dT)₁₈ standard using the following equation:

$$(N/L) = (5.54/L) \left(\frac{t_R}{w_{0.5}} \right)^2$$

wherein N is the number of theoretical plates, t_R is the retention time of said standard determined during an isocratic elution, $w_{0.5}$ is the peak width at half height, and L is the length of the monolith in meters.

25. A method of claim 23 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like
26. A method of claim 23 wherein said tube is silianized.
27. A method of claim 23 wherein said tube is devoid of retaining frits.
28. A method of claim 23 wherein said mobile phase is devoid of EDTA.
29. A method of claim 23 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.
30. A method for separating a mixture of polynucleotides, said method comprising: applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, wherein said mobile phase is devoid of EDTA, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix
31. A method of claim 30 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface

morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is brush-like.

32. A method of claim 30 wherein said monolith is immobilized by covalent
attachment at the inner wall of said tube.

5 33. A method of claim 32 wherein said tube is devoid of retaining frits.

34. A method of claim 30 wherein said monolith is characterized by having 10,000 to
200,000 theoretical plates per meter.

10 35. A method of claim 30 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is rugulose.

36. A method of claim 30 wherein said tube has been silanized.

15 37. A method for separating a mixture of polynucleotides, said method comprising:
applying said mixture of polynucleotides to a polymeric monolith having non-
polar chromatographic surfaces and eluting said mixture of polynucleotides
with a mobile phase comprising a counterion agent and an organic solvent,
wherein said monolith comprises an underivatized poly(styrene-
divinylbenzene) matrix,
20 wherein said monolith has a surface morphology, as determined by scanning
electron microscopy, that resembles the surface morphology of octadecyl
modified poly(styrene-divinylbenzene) particles, wherein said surface
morphology of said monolith is rugulose.

38. A method of claim 37 wherein said mobile phase is devoid of EDTA.

25 39. A method of claim 37 wherein said monolith is contained within a fused silica
tube having an inner diameter in the range of 1 micrometer to 1000
micrometer.

40. A method of claim 37 wherein said monolith is immobilized by covalent
attachment at the inner wall of said tube.

30 41. A method of claim 37 wherein said tube is devoid of retaining frits.

42. A method of claim 37 wherein said monolith is characterized by having 100,000
to 200,000 theoretical plates per meter.

43. A method of claim 37 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface

morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is brush-like.

44. A method for separating a mixture of polynucleotides, said method comprising:
applying said mixture of polynucleotides to a polymeric monolith having non-
polar chromatographic surfaces and eluting said mixture of polynucleotides
with a mobile phase comprising a counterion agent and an organic solvent,
wherein said monolith comprises an underivatized poly(styrene-
divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube having an inner
diameter in the range of 1 micrometer to 1000 micrometer,
wherein said monolith is immobilized at the inner wall of said tube,
wherein said tube is devoid of retaining frits.

45. A method of claim 44 wherein said mobile phase is devoid of EDTA.

46. A method of claim 44 wherein said monolith is contained within a tube having an
inner diameter in the range of 10 micrometer to 300 micrometer.

47. A method of claim 44 wherein said monolith is immobilized at the inner wall of
said tube and wherein said tube has been silanized.

48. A method of claim 44 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is brush-like:

49. A method of claim 44 wherein said monolith is characterized by having 100,000
to 200,000 theoretical plates per meter.

50. A method of claim 44 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is rugulose.

51. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-
divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

52. A device of claim 51 wherein said tube is devoid of retaining frits.

5 53. A device of claim 51 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

54. A device of claim 53 wherein said theoretical plates per meter is determined from the retention time of single stranded p(dT)₁₈ standard using the following equation:

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$$(N/L) = (5.54/L) \left(\frac{t_R}{w_{0.5}} \right)^2$$

wherein N is the number of theoretical plates, t_R is the retention time of said standard determined during an isocratic elution, $w_{0.5}$ is the peak width at half height, and L is the length of the monolith in meters.

55. A device of claim 54 wherein said tube has an inner diameter of 200 micrometer and a length of 60 mm, wherein during said isocratic elution said monolith has a back pressure in the range of 180 to 200 bar, and a flow rate in the range of 2 to 3 $\mu\text{L}/\text{min}$ at an elution temperature of 50°C.

56. A device of claim 51 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

57. A device of claim 51 wherein the chromatographic surfaces of said monolith are devoid of micropores.

58. A device of claim 57 wherein said monolith has channels sufficiently large for convective flow of said mobile phase.

59. A device for separating a mixture of polynucleotides, said device comprising: a polymeric monolith having non-polar chromatographic surfaces, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix, wherein said monolith is contained within a fused silica tube, and

wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

60. A device of claim 59 wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer.

5 61. A device of claim 59 wherein said tube is devoid of retaining frits.

62. A device of claim 59 wherein said monolith is characterized by having 10,000 to 200,000 theoretical plates per meter.

63. A device of claim 59 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

64. A device of claim 59 wherein said monolith comprises an underivatized monolithic stationary phase.

65. A device of claim 59 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

66. A device of claim 59 wherein said monolith is devoid of micropores and wherein said monolith has channels sufficiently large for convective flow of said mobile phase.

67. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,
wherein said monolith is contained within a fused silica tube,
wherein said tube has been silanized, and
wherein said tube is devoid of retaining frits.

68. A device of claim 67 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

69. A device of claim 67 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

70. A device of claim 67 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface

morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is brush-like.

71. A device of claim 67 wherein said tube has an inner diameter in the range of 1
micrometer to 1000 micrometer.

5 72. A device of claim 67 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is rugulose.

10 73. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-
divinylbenzene) matrix,
wherein said monolith is contained within a tube having an inner diameter in
15 the range of 1 micrometer to 1000 micrometer,
wherein said monolith is characterized by having 10,000 to 200,000
theoretical plates per meter.

74. A device of claim 73 wherein said monolith is contained within a tube having an
inner diameter in the range of 1 micrometer to 1000 micrometer.

20 75. A device of claim 73 wherein said monolith is immobilized by covalent
attachment at the inner wall of said tube.

76. A device of claim 75 wherein said tube is devoid of retaining frits.

25 77. A method of claim 73 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is brush-like.

30 78. A method of claim 73 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles,
wherein said surface morphology of said monolith is rugulose.

79. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is characterized by having at least 100,000 theoretical plates per meter,

5 wherein said monolith is contained within a silanized fused silica tube having an inner diameter in the range of 10 micrometer to 1000 micrometer, wherein said monolith is immobilized at the inner wall of said tube.

80. A device of claim 79 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

10 81. A device of claim 79 wherein said monolith is contained within a tube having an inner diameter in the range of 1 micrometer to 1000 micrometer.

82. A device of claim 79 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

83. A device of claim 82 wherein said tube is devoid of retaining frits.

84. A device of claim 79 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

85. A miniaturized chromatographic system for separating a mixture of polynucleotides, said system comprising the device of claim 79.

86. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
25 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose and brush-like,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,
30 wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer,
wherein said monolith is immobilized at the inner wall of said tube.

87. A device of claim 86 wherein said tube is devoid of retaining frits.

88. A device of claim 86 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

89. A device of claim 86 wherein said tube has been silanized.

90. A device of claim 86 wherein said surfaces of said monolith are non-porous.

5 91. A device of claim 86 wherein said monolith is formed from a polymerization mixture including underivatized styrene, a crosslinking agent, and a porogen, wherein said porogen comprises tetrahydrofuran.

92. A device of claim 86 wherein said polynucleotides comprise double-stranded fragments having lengths in the range of 3 to 600 base pairs.

10 93. A method of claim 16 including analyzing eluted polynucleotides by mass spectral analysis.

94. A method of claim 23 including analyzing eluted polynucleotides by mass spectral analysis.

95. A system of claim 85 wherein said monolith is operatively coupled to a mass spectrometer.

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15 96. A method for desalting a mixture of polynucleotides, said method comprising: applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix, wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, wherein said monolith is immobilized at the inner wall of said tube.

25 97. A chromatographic device, said device comprising: a polymeric monolith having non-polar chromatographic surfaces, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix, wherein said monolith is contained within a silanized fused silica tube having an inner diameter in the range of 10 micrometer to 1000 micrometer, and wherein said monolith is immobilized at the inner wall of said tube.

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